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IN RE APPLICATION OF: Fabrizio SAMARITANI, et al.

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FOR: LIQUID PHARMACEUTICAL FORMULATIONS OF FSH AND LH TOGETHER WITH A NON-IONIC SURFACTANT

REQUEST FOR PRIORITY UNDER 35 U.S.C. 119 AND THE INTERNATIONAL CONVENTION

Commissioner for Patents
Alexandria, Virginia 22313

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

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Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. PCT/EP04/50432.

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Bescheinigung Certificate Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

03100882.4

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

FSH AND FSH VARIANT FORMULATIONS

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FSH and FSH variant formulations

Field of Invention

The invention relates to the field of pharmaceutical formulations of follicle-stimulating hormone (FSH), and to methods of producing such formulations.

Background of the invention

Follicle-stimulating hormone (FSH) is an injectable protein falling into the class of gonadotrophins, which includes FSH, luteinising hormone (LH) and chorionic gonadotrophin (CG). FSH is used in the treatment of infertility in both female and male patients.

FSH and its variants may be produced recombinantly (recombinant FSH or rFSH), or FSH may be produced from the urine of postmenopausal women (urinary FSH or uFSH).

FSH is used in female patients in ovulation induction (OI) and in controlled ovarian hyperstimulation (COH) for assisted reproductive technologies (ART). In a typical treatment regimen for ovulation induction, a patient is administered daily injections of FSH or a variant (about 75 to 300 IU FSH/day) for a period of from about 6 to about 12 days. In a typical treatment regimen for controlled ovarian hyperstimulation, a patient is administered daily injections of FSH or a variant (about 150-600 IU FSH/day) for a period of from about 6 to about 12 days.

FSH is also used to induce spermatogenesis in men suffering from oligospermia. A regimen using 150 IU FSH 3 times weekly in combination with 2'500 IU hCG twice weekly has been successful in achieving an improvement in sperm count in men suffering from hypogonadotrophic hypogonadism [1].

FSH is a member of the heterodimer, glycoprotein hormone family that includes thyroid stimulating hormone (TSH), chorionic gonadotrophin (CG), and luteinising hormone (LH). The members of this family are heterodimers, comprising an α- and a β-subunit. The subunits are held together by noncovalent interactions. The human FSH (hFSH) heterodimer consists of (i) a mature 92 amino acid alpha subunit, which also is common to the other human family members (i.e., chorionic gonadotrophin ("CG"), luteinising hormone ("LH") and thyroid stimulating hormone ("TSH"); and (ii) a

mature 111 amino acid beta subunit that is unique to FSH[2]. The alpha and beta subunits may be prone to dissociate in formulations, due to interaction with the preservative, surfactant and other excipients. Dissociation of the subunits leads to loss of potency.

FSH is formulated for intramuscular (IM) or subcutaneous (SC) injection. It is supplied in a lyophilised (solid) form in vials or ampoules of 75 IU/vial and 150 IU/vial with a shelf life of one and a half to two years when stored at 2-25°C. A solution for injection is formed by reconstituting the lyophilised product with water for injection (WFI). For ovulation induction or controlled ovarian hyperstimulation, daily injections with starting doses of 75 IU to 600 IU are recommended for up to about ten days. Depending on the patient's response, up to three cycles of treatment with increasing doses of FSH can be used. With lyophilised formulations, the patient is required to reconstitute a new vial of lyophilised material with diluent and administer it immediately after reconstitution on a daily basis [Package insert N1700101A, published in February 1996, for Fertinex™ (urofollitropin for injection, purified) for subcutaneous injection, by Serono Laboratories, Inc., Randolph, MA].

FSH has also been formulated in both single-dose and multi-dose liquid formats, in vials, or ampoules. Single dose formats must remain stable and potent in storage prior to use. Multi-dose formats must not only remain stable and potent in storage prior to use, but must also remain stable, potent and relatively free of bacteria over the multiple use regimen administration period, after the seal of the ampoule has been compromised. For this reason, multi-dose formats often contain a bacteriostatic agent.

EP 0 618 808 (Applied Research Systems ARS Holding N.V.) discloses a pharmaceutical composition comprising a solid intimate mixture of gonadotrophin and a stabilising amount of sucrose alone or in combination with glycine.

EP 0 814 841 (Applied Research Systems ARS Holding N.V.) discloses a stable, liquid pharmaceutical composition comprising recombinant human chorionic gonadotrophin (hCG) and a stabilizing amount of mannitol.

EP 0 448 146 (AKZO N.V.) discloses a stabilized gonadotrophin containing lyophilisate comprising one part by weight of a gonadotrophin; and 200 to 10,000

parts by weight of a dicarboxylic acid salt stabilizer associated with the gonadotrophin.

EP 0 853 945 (Akzo Nobel N.V.) discloses a liquid gonadotrophin-containing formulation characterised in that the formulation comprises a gonadotrophin and stabilising amounts of a polycarboxylic acid or a salt thereof and of a thioether compound.

WO 00/04913 (Eli Lilly and Co.) discloses a formulation comprising FSH or an FSH variant, containing an alpha and beta subunit, and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent.

There remains a need for stable liquid formulations of FSH or FSH variants, either for single dose or multiple dose administration.

Summary of the invention

It is an object of the invention to provide new liquid formulations of FSH or FSH variants, to provide methods for their preparation, and methods for their pharmaceutical or veterinary use in the treatment of fertility disorders.

In a first aspect, the invention provides a liquid pharmaceutical composition comprising FSH or a variant thereof, and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

In a second aspect, the invention provides a method for manufacturing a liquid pharmaceutical composition comprising forming a solution of FSH or a variant thereof, and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 and a suitable diluent.

In a third aspect, the invention provides a method for manufacturing a packaged pharmaceutical composition comprising placing a solution comprising FSH, and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

In a fourth aspect, the invention provides an article of manufacture for human pharmaceutical use, comprising a vial comprising a solution of FSH or an FSH variant, and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 and written material stating that such solution may be held over a period of at or about twenty-four hours or greater after the first use.

Detailed description of the invention

Brief description of the drawings

Figure 1 shows the percentage of oxidised α-subunit in formulations of FSH containing Pluronic F68, methionine at 10 µg/ml ("Meth 10 mcg/ml") and 100 µg/ml ("Meth 100 mcg/ml") versus a formulation with no methionine ("No methionine"), at time 0, 1 week and 2 weeks.

The FSH or FSH variant solutions and formulations of the invention have improved or more suitable properties or stability, and are useful for infertility treatment in women and/or men. These formulations and articles of manufacture are additionally suitable for use in injectable and alternative delivery systems, e.g., but not limited to, nasal, pulmonary, transmucosal, transdermal, oral, subcutaneous, intramuscular or parenteral sustained release. In a particularly preferred embodiment the formulations of the invention are for subcutaneous and/or intramuscular injection. The FSH or FSH variant solutions and formulations provided may also have increased *in vivo* potency compared to known commercial products, by preventing or reducing loss of activity or stability, or by improving any aspect of the effectiveness or desirability of administration, e.g., by at least one of mode, frequency, dosage, comfort, ease of use, biological activity *in vitro* or *in vivo,* and the like.

Follicle stimulating hormone, or FSH, as used herein refers to the FSH produced as a full-length mature protein which includes, but is not limited to human FSH or "hFSH", whether produced recombinantly or isolated from human sources, such as the urine of postmenopausal women. The protein sequence of the human FSH alpha subunit is provided in SEQ ID NO: 1, and the protein sequence of the human FSH beta subunit is given in SEQ ID NO:2.

The expression "FSH variant" is meant to encompass those molecules differing in amino acid sequence, glycosylation pattern or in inter-subunit linkage from human FSH but exhibiting FSH-activity. Examples include CTP-FSH, a long-acting modified recombinant FSH, consisting of the wild type α-subunit and a hybrid β-subunit in

which the carboxy terminal peptide of hCG has been fused to the C-terminal of the β-subunit of FSH, as described in LaPolt *et al.*; Endocrinology; **1992**, *131*, 2514-2520; or Klein et al.; Development and characterization of a long-acting recombinant hFSH agonist; Human Reprod. 2003, 18, 50-56]. Also included is single chain CTP-FSH, a single chain molecule, consisting of the following sequences (from N-terminal to C-terminal):

βFSH	βhCG-CTP(113-145)	αFSH

wherein βFSH signifies the β-subunit of FSH, βhCG CTP (113-145) signifies the carboxy terminal peptide of hCG and αFSH signifies the α-subunit of FSH, as described by Klein *et al.*[3] Other examples of FSH variants include FSH molecules having additional glycosylation sites incorporated in the α- and/or β-subunit, as disclosed in WO 01/58493 (Maxygen), particularly as disclosed in claims 10 and 11 of WO 01/58493, and FSH molecules with intersubunit S-S bonds, as disclosed in WO 98/58957.

The FSH variants referred to herein also include the carboxy terminal deletions of the beta subunit that are shorter than the full length mature protein of SEQ ID NO:2. Carboxy terminal deletions of the human beta subunit are provided in SEQ IDS NOS: 3, 4, and 5. It is understood that the carboxy terminal variants of the beta chain form dimers with a known alpha subunit to form an FSH variant heterodimer.

FSH heterodimers or FSH variant heterodimers can be produced by any suitable method, such as recombinantly, by isolation or purification from natural sources as may be the case, or by chemical synthesis, or any combination thereof.

The use of the term "recombinant" refers to preparations of FSH or FSH variants that are produced through the use of recombinant DNA technology (see for example WO 85/01958). The sequences for genomic and cDNA clones are known for the alpha and beta subunits of several species[4]. Transfection of eukaryotic cells with the DNA sequences encoding a alpha and beta subunit, whether provided on one vector or on two vectors with each subunit having a separate promoter are capable of providing intact dimers.

The FSH or FSH variant used in accordance with the present invention may be produced not only by recombinant means, including from mammalian cells, but also may be purified from other biological sources, such as from urinary sources. Acceptable methodologies include those described in Hakola, K. Molecular and Cellular Endocrinology, 127:59-69, 1997; Keene, et al., J. Biol. Chem., 264:4769-4775, 1989; Cerpa-Poljak, et al., Endocrinology, 132:351-356, 1993; Dias, et al., J. Biol. Chem., 269:25289-25294, 1994; Flack, et al., J. Biol. Chem., 269:14015-14020, 1994; and Valove, et al., Endocrinology, 135:2657-2661, 1994, U.S. Patent 3,119,740 and US Patent no. 5,767,067.

The term "administer" or "administering" means to introduce a formulation of the present invention into the body of a patient in need thereof to treat a disease or condition.

The term "patient" means a mammal that is treated for a disease or condition. Patients are of, but not limited to, the following origin, human, ovine, porcine, equine, bovine, rabbit and the like.

The term "potency" refers to the ability of an FSH formulation to elicit biological responses associated with FSH, such as ovarian weight gain in the Steelman-Pohley assay[5], or follicular growth in a female patient. Follicular growth in a female patient can be evaluated by ultrasound, for example, in terms of the number of follicles having a mean diameter of at or about 16 mm on day 8 of stimulation.

The term "aqueous diluent" refers to a liquid solvent that contains water. Aqueous solvent systems may be consist solely of water, or may consist of water plus one or more miscible solvents, and may contain dissolved solutes such as sugars, buffers, salts or other excipients. The more commonly used non-aqueous solvents are the short-chain organic alcohols, such as, methanol, ethanol, propanol, short-chain ketones, such as acetone, and poly Alcohols, such as glycerol.

An "isotonicity agent" is a compound that is physiologically tolerated and imparts a suitable tonicity to a formulation to prevent the net flow of water across cell membranes that are in contact with the formulation. Compounds such as glycerin, are commonly used for such purposes at known concentrations. Other suitable isotonicity agents include, but are not limited to, amino acids or proteins (e.g., glycine

or albumin), salts (e.g., sodium chloride), and sugars (e.g., dextrose, sucrose and lactose).

The term "bacteriostatic" refers to a compound or compositions added to a formulation to act as an anti-bacterial agent. A preserved FSH or FSH variant containing formulation of the present invention preferably meets statutory or regulatory guidelines for preservative effectiveness to be a commercially viable multi-use product. Examples of bacteriostatics include phenol, *m*-cresol, *p*-cresol, *o*-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal.

The term "buffer" or "physiologically-acceptable buffer" refers to solutions of compounds that are known to be safe for pharmaceutical or veterinary use in formulations and that have the effect of maintaining or controlling the pH of the formulation in the pH range desired for the formulation. Acceptable buffers for controlling pH at a moderately acidic pH to a moderately basic pH include, but are not limited to, such compounds as phosphate, acetate, citrate, arginine, TRIS, and histidine. "TRIS" refers to 2-amino-2-hydroxymethyl-1,3,-propanediol, and to any pharmacologically acceptable salt thereof. Preferable buffers are phosphate buffers with saline or an acceptable salt.

The term "phosphate buffer" refers to solutions containing phosphoric acid or salts thereof, adjusted to a desired pH. Generally phosphate buffers are prepared from phosphoric acid, or a salt of phosphoric acid, including but not limited to sodium and potassium salts. Several salts of phosphoric acid are known in the art, such as sodium and potassium monobasic, dibasic, and tribasic salts of the acid. Salts of phosphoric acid are also known to occur as hydrates of the occurring salt. Phosphate buffers may cover a range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of at or about 6.0 to at or about 8.0, most preferably at or about pH 7.0.

The term "vial" refers broadly to a reservoir suitable for retaining FSH in solid or liquid form in a contained sterile state. Examples of a vial as used herein include ampoules, cartridges, blister packages, or other such reservoir suitable for delivery of the FSH to the patient via syringe, pump (including osmotic), catheter, transdermal patch, pulmonary or transmucosal spray. Vials suitable for packaging products for

parenteral, pulmonary, transmucosal, or transdermal administration are well known and recognized in the art.

The term "stability" refers to the physical, chemical, and conformational stability of formulations of FSH of the present invention (including maintenance of biological potency). Instability of a protein formulation may be caused by chemical degradation or aggregation of the protein molecules to form higher order polymers, by dissociation of the heterodimers into monomers, deglycosylation, modification of glycosylation, oxidation (particularly of the α-subunit) or any other structural modification that reduces at least one biological activity of an FSH polypeptide included in the present invention.

A "stable" solution or formulation, is one wherein the degree of degradation, modification, aggregation, loss of biological activity and the like, of proteins therein is acceptably controlled, and does not increase unacceptably with time. Preferably the formulation retains at least at or about 60%, more preferably at least at or about 70 %, most preferably at least at or about 80% of the labelled FSH activity over a period of 24 months. FSH activity can be measured using the Steelman-Pohley ovarian weight gain bioassay[5].

The term "treating" refers to the administration, follow up, management and/or care of a patient for which FSH administration is desirable for the purpose of follicle or testicular stimulation or any other physiological response regulated by FSH. Treating can thus include, but is not limited to, the administration of FSH for the induction or improvement of sperm or follicular development or for ovulation induction.

The expression "multi-dose use" is intended to include the use of a single vial, ampoule or cartridge of an FSH formulation for more than one injection, for example 2, 3, 4, 5, 6 or more injections. The injections are preferably made over a period of at least at or about 12 hours, 24 hours, 48 hours, etc., preferably up to a period of at or about 12 days. The injections may be spaced in time, for example, by a period of 6, 12, 24, 48 or 72 hours.

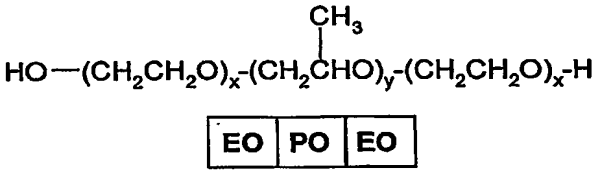
A " salt" of a protein is an acid or base addition salt. Such salts are preferably formed between any one or more of the charged groups in the protein and any one or more physiologically acceptable, non-toxic cations or anions. Organic and inorganic salts include, for example, those prepared from acids such as hydrochloric, sulphuric,

sulfonic, tartaric, fumaric, hydrobromic, glycolic, citric, maleic, phosphoric, succinic, acetic, nitric, benzoic, ascorbic, p-toluenesulfonic, benzenesulfonic, naphthalenesulfonic, propionic, carbonic, and the like, or for example, ammonium, sodium, potassium, calcium, or magnesium.

The inventors have found that by formulating FSH with a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic® F68, particularly preferably Pluronic F68 (BASF, Pluronic F68 is also known as Poloxamer 188) they obtain stable formulations that minimise the loss of active principle (FSH) caused by adsorption on the surfaces of the vial and/or delivery device (e.g. syringe, pump, catheter, etc.).

The inventors have further found that by formulating FSH with a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic® F68, particularly preferably Pluronic F68 (BASF, Pluronic F68 is also known as Poloxamer 188) they obtain a stable formulation that avoids the problem of precipitation in the presence of a bacteriostatic agent, such as *m*-cresol and phenol. Precipitation, resulting in the formation of turbid or milky solutions occurs when TWEEN 20 is used with m-cresol or phenol.

The Pluronic surfactants are block copolymers of ethylene oxide (EO) and propylene oxide (PO). The propylene oxide block (PO) is sandwiched between two ethylene oxide (EO) blocks.



Pluronic surfactants are synthesised in a two-step process:

1. A hydrophobe of the desired molecular weight is created by the controlled addition of propylene oxide to the two hydroxyl groups of propylene glycol; and

2. Ethylene oxide is added to sandwich the hydrophobe between hydrophilic groups.

In Pluronic® F77, the percentage of polyoxyethylene (hydrophile) is 70%, and the molecular weight of the hydrophobe (polyoxypropylene) is approximately 2,306 Da.

In Pluronic F87, the percentage of polyoxyethylene (hydrophile) is 70%, and the molecular weight of the hydrophobe (polyoxypropylene) is approximately 2,644 Da.

In Pluronic F88, the percentage of polyoxyethylene (hydrophile) is 80%, and the molecular weight of the hydrophobe (polyoxypropylene) is approximately 2,644 Da.

In Pluronic F68, the percentage of polyoxyethylene (hydrophile) is 80%, and the molecular weight of the hydrophobe (polyoxypropylene) is approximately 1,967 Da.

Typical properties of Pluronic F77 are listed below:
Average Molecular Weight: 6600;
Melt/pour point: 48°C ;
Physical Form @ 20°C : solid;
Viscosity (Brookfield) cps: 480 [liquids at 25°C, pastes at 60°C and solids at 77°C];
Surface tension, dynes/cm @ 25°C;
0.1% Conc. : 47.0
0.01% Conc. : 49.3
0.001% Conc.: 52.8
Interfacial tension, dynes/cm @ 25°C vs. Nujol;
0.1% Conc. : 17.7
0.01% Conc. : 20.8
0.01% Conc. : 25.5
Draves Wetting, Seconds 25°C
1.0% Conc.: > 360
0.1% Conc.: > 360
Foam Height
Ross Miles, 0.1%, mm @ 50°C: 100
Ross Miles, 0.1%, mm @ 26°C: 47
Dynamic, 0.1%, mm @ 400 ml/min: > 600
Cloud point in aqueous solution, °C
1% Conc.: >100
10% Conc.: >100
HLB (hydrophile-lipophile balance): 25

Typical properties of Pluronic F87 are listed below:
Average Molecular Weight: 7700;
Melt/pour point: 49°C ;
Physical Form @ 20°C : solid;
Viscosity (Brookfield) cps: 700 [liquids at 25°C, pastes at 60°C and solids at 77°C];
Surface tension, dynes/cm @ 25°C;
0.1% Conc. : 44.0
0.01% Conc. : 47.0
0.001% Conc.: 50.2
Interfacial tension, dynes/cm @ 25°C vs Nujol;
0.1% Conc. : 17.4
0.01% Conc. : 20.3
0.01% Conc. : 23.3
Draves Wetting, Seconds 25°C

1.0% Conc.: > 360
0.1% Conc.: > 360
Foam Height
Ross Miles, 0.1%, mm @ 50°C: 80
Ross Miles, 0.1%, mm @ 26°C: 37
Dynamic, 0.1%, mm @ 400 ml/min: > 600
Cloud point in aqueous solution, °C
1% Conc.: >100
10% Conc.: >100
HLB (hydrophile-lipophile balance): 24

Typical properties of Pluronic F88 are listed below:
Average Molecular Weight: 11400;
Melt/pour point: 54°C ;
Physical Form @ 20°C : solid;
Viscosity (Brookfield) cps: 2300 [liquids at 25°C, pastes at 60°C and solids at 77°C];
Surface tension, dynes/cm @ 25°C;
0.1% Conc. : 48.5
0.01% Conc. : 52.6
0.001% Conc.: 55.7
Interfacial tension, dynes/cm @ 25°C vs Nujol;
0.1% Conc. : 20.5
0.01% Conc. : 23.3
0.01% Conc. : 27.0
Draves Wetting, Seconds 25°C
1.0% Conc.: > 360
0.1% Conc.: > 360
Foam Height
Ross Miles, 0.1%, mm @ 50°C: 80
Ross Miles, 0.1%, mm @ 26°C: 37
Dynamic, 0.1%, mm @ 400 ml/min: > 600
Cloud point in aqueous solution, °C
1% Conc.: >100
10% Conc.: >100
HLB (hydrophile-lipophile balance): 28

Typical properties of Pluronic F68 are listed below:
Average Molecular Weight: 8400;
Melt/pour point: 52°C ;
Physical Form @ 20°C : solid;
Viscosity (Brookfield) cps: 1000 [liquids at 25°C, pastes at 60°C and solids at 77°C];
Surface tension, dynes/cm @ 25°C;
0.1% Conc. : 50.3
0.01% Conc. : 51.2
0.001% Conc.: 53.6
Interfacial tension, dynes/cm @ 25°C vs Nujol;
0.1% Conc. : 19.8
0.01% Conc. : 24.0
0.01% Conc. : 26.0
Draves Wetting, Seconds 25°C
1.0% Conc.: > 360
0.1% Conc.: > 360
Foam Height
Ross Miles, 0.1%, mm @ 50°C: 35
Ross Miles, 0.1%, mm @ 26°C: 40

Dynamic, 0.1%, mm @ 400 ml/min: > 600
Cloud point in aqueous solution, °C
1% Conc.: >100
10% Conc.: >100
HLB (hydrophile-lipophile balance): 29

Other polymers having properties similar to those listed above may also be used in the formulations of the invention. The preferred surfactant is Pluronic F68, and surfactants having similar properties.

Pluronic, particularly Pluronic F68, is preferably present at a concentration that is sufficient to maintain FSH stability over the desired storage period (for example 12 to 24 months), and also at a concentration that is sufficient to prevent protein losses due to adsorption on surfaces, such as the vial, ampoule or cartridge or the syringe. Preferably the concentration of Pluronic, particularly Pluronic F68, in liquid formulations is at or about 0.01 mg/ml to at or about 1 mg/ml, more preferably at or about 0.05 mg/ml to at or about 0.5 mg/ml, more particularly preferably at or about 0.2 mg/ml to at or about 0.4 mg/ml, most preferably at or about 0.1 mg/ml.

Preferably the concentration of FSH in the formulation is at or about 150 IU/ml to at or about 1'200 IU/ml, more preferably at or about 300 IU/ml to at or about 900 IU/ml, more particularly preferably at or about 450 to at or about 750, most preferably at or about 600 IU/ml.

Preferably the formulations of the present invention have pH between at or about 6.0 and at or about 8.0, more preferably at or about 6.8 to at or about 7.8, including about pH 7.0, pH 7.2, and 7.4. A preferred buffer is phosphate, with preferred counterions being sodium or potassium ions. Phosphate saline buffers are well known in the art, such as Dulbecco's Phosphate buffered saline. Buffer concentrations in total solution can vary between at or about 5mM, 9.5mM, 10mM, 50mM, 100mM, 150mM, 200mM, 250mM, and 500mM. Preferably the buffer concentration is at or about 10mM. Particularly preferred is a buffer 10 mM in phosphate ions with a pH of 7.0.

The invention includes liquid formulations. The preferred solvent is water for injection.

Liquid formulations may be single dose or multi-dose. Those liquid FSH formulations of the invention that are intended for multi-dose use preferably comprise a

bacteriostatic, such as phenol, *m*-cresol, *p*-cresol, *o*-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal. Particularly preferred are phenol, benzyl alcohol and *m*-cresol, more preferred are phenol and *m*-cresol, most preferred is *m*-cresol. The bacteriostatic agent is used in an amount that will yield a concentration that is effective to maintain the formulation essentially bacteria free (suitable for injection) over the multi-dose injection period, which may be at or about 12 or 24 hours to at or about 12 days, preferably at or about 6 to at or about 12 days. The bacteriostatic is preferably present in a concentration of at or about 0.1% (mass bacteriostatic/mass of solvent) to at or about 2.0%, more preferably at or about 0.2% to at or about 1.0%. In the case of benzyl alcohol, particularly preferred is a concentration of 0.9%). In the case of phenol, particularly preferred is at or about 0.5%. In the case of *m*-cresol, particularly preferred is a concentration of at or about 0.3 % (e.g. at or about 3 mg/ml in WFI).

In a preferred embodiment, the invention provides a liquid pharmaceutical composition comprising FSH or a variant thereof, a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and a bacteriostatic selected from m-cresol and phenol, preferably m-cresol.

In a further preferred embodiment, the invention provides a method for manufacturing a liquid pharmaceutical composition comprising forming a solution of FSH or a variant thereof, a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and a bacteriostatic selected from m-cresol and phenol, preferably m-cresol, and a suitable diluent.

In yet another preferred embodiment, the invention provides a method for manufacturing a packaged pharmaceutical composition comprising placing a solution comprising FSH, a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and a bacteriostatic selected from m-cresol and phenol, preferably m-cresol.

In yet another preferred embodiment, the invention provides an article of manufacture for human pharmaceutical use, comprising a vial comprising a solution of FSH or an FSH variant, a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and a bacteriostatic selected from m-cresol and phenol, preferably m-cresol, and written material stating that such solution may be

held over a period of at or about twenty-four hours or greater after the first use. Preferably the written material states that the solution may be held up to at or about 12 days.

In a particularly preferred embodiment, the formulation comprises *m*-cresol and Pluronic F68. The inventors have surprisingly found that formulations comprising Pluronic F68 do not precipitate in the presence of *m*-cresol, a problem observed with other surfactants.

After the first use of a multi-dose formulation it may be kept and used for at least at or about 24 hours, preferably at least at or about 4, 5 or 6 days, more preferably for up to 12 days. After the first use the formulation it is preferably stored at below room temperature (i.e. below at or about 25°C), more preferably below at or about 10°C, more preferably at or about 2-8°C, most preferably at or about 5-0°C.

Preferably the formulations of the invention contain an antioxidant, such as methionine, sodium bisulfite, salts of ethylenediaminetetraacetic acid (EDTA), butylated hydroxytoluene (BHT), and butylated hydroxy anisole (BHA). Most preferred is methionine. The antioxidant prevents oxidation of the FSH (particularly the α-subunit), which leads to loss of potency.

Methionine is preferably present at a concentration of at or about 0.01 to at or about 1.0 mg/ml, more preferably at or about 0.05 to at or about 0 .5 mg/ml, most preferably at or about 0.1 mg/ml. Also preferably, methionine is present in an amount in mg that is at or about the total FSH content in IU divided by 6'000, as calculated according to equation (2):

$$\text{Amount of methionine} = \frac{\text{Total FSH in IU}}{\text{6'000 IU/mg}} \qquad (2)$$

(i.e. 0.05 mg methionine for 300 IU FSH, 0.075 mg methionine for 450 IU FSH, etc.).

Preferably the formulations of the invention contain a mono- or disaccharide or a sugar alcohol as stabiliser and tonicity adjusting agent, such as sucrose, dextrose, lactose, mannitol and/or glycerol. Most preferred is sucrose, preferably at a

concentration of 60 mg/ml, and also preferably in an amount in mg that is at or about the total content of FSH in IU divided by 10, as calculated according to equation (3):

$$\text{Amount of sucrose} = \frac{\text{Total FSH in IU}}{\text{10 IU/mg}} \qquad (3)$$

(i.e. 30 mg of sucrose for 300 IU FSH, 45 mg sucrose for 450 IU FSH, etc.).

As noted above, the invention provides formulations for single use and formulations for multi-dose use, containing a bacteriostatic, or to which a bacteriostatic is added when the formulation is reconstituted. The formulations of the invention are suitable for pharmaceutical or veterinary use, comprising FSH or FSH variant in a pharmaceutically acceptable formulation.

As noted above, the invention provides an article of man ufacture, comprising packaging material and a vial comprising a solution of FSH or an FSH variant, Pluronic F68 and a bacteriostatic selected from phenol and *m*-cresol, optionally with buffers and/or other excipients, optionally in an aqueous diluent, where in said packaging material comprises written material which indicates that such solution may be held over a period of twenty-four hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a vial comprising a formulation of FSH or an FSH variant according to the invention, wherein said packaging material comprises written material which instructs a patient to reconstitute the FSH or an FSH variant in the aqueous diluent to form a solution which may be held over a period of twenty-four hours or greater.

The range of protein hormone in the formulations of the invention includes amounts yielding upon reconstitution, concentrations from about 1.0 µg/ml to about 50 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods. The protein hormone concentration is preferably at or about 5.0 µg/ml to at or about 2 mg/ml, more preferably at or about 10 µg/ml to at or about 1 mg/ml, most preferably at or about 50 µg/ml to at or about 200 µg/ml.

Preferably the formulations of the invention retain at least at or about 60%, more preferably at least at or about 70 %, most preferably at least at or about 80% of the FSH activity at the time of packaging over a period of 24 months. FSH activity can be measured using the Steelman-Pohley ovarian weight gain bioassay[5].

The formulations of the present invention can be prepared by a process which comprises mixing FSH or an FSH variant and Pluronic F68 and a bacteriostatic selected from phenol and *m*-cresol as solids or dissolving FSH or an FSH variant and Pluronic F68 and a bacteriostatic selected from phenol and *m*-cresol in an aqueous diluent. Mixing the components and dissolving them in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of FSH or an FSH variant in buffered solution is combined with Pluronic F68 and a bacteriostatic selected from phenol and *m*-cresol in a buffered solution in quantities sufficient to provide the protein, Pluronic F68 and the bacteriostatic at the desired concentrations. The resulting solution is then placed in vials, ampoules or cartridges. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that may be optimised for the concentration and means of administration used.

The formulations of the invention can be administered using recognized devices. Examples comprising these single vial systems include pen-injector devices for delivery of a solution such as EasyJect®, Gonal-F® Pen, Humaject®, NovoPen®, B-D®Pen, AutoPen®, and OptiPen®.

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product may be used. The packaging material of the present invention provides instructions to the patient to reconstitute the FSH or an FSH variant in the aqueous diluent to form a solution and to use the solution over a period of twenty-four hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution may be used over a period of twenty-four hours or greater. The presently claimed products are useful for human pharmaceutical product use.

The stable preserved formulations may be provided to patients as clear solutions. The solution may be for single use or it may be reused multiple times and may suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

FSH or an FSH variant in either the stable or preserved formulations or solutions described herein, may be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, oral, or other means appreciated by the skilled artisan, as well-known in the art.

The following examples are provided merely to further illustrate the preparation of the formulations and compositions of the invention. The scope of the invention shall not be construed as merely consisting of the following examples.

Example 1

Comparative formulations

Materials

Item	Manufacturer
r-hFSH Bulk used for candidate formulations	Laboratoires Serono SA
D-Mannitol (DAB, Ph Eur, BP, FU, USP, FCC, E421)	Merck
Sucrose (DAB, Ph Eur, BP, NF)	Merck
NaCl (ACS, ISO)	Merck
$Na_2HPO_4\ 2H_2O$ (GR for analysis)	Merck
$NaH_2PO_4\ H_2O$ (GR for analysis)	Merck
Benzyl Alcohol (GR for analysis)	Merck
m-Cresol (for synthesis)	Merck
TWEEN 20 (Polysorbate 20) (for synthesis)	Merck
Pluronic F68 (Poloxamer 188)	Sigma
l-Methionine (for biochemistry)	Merck
Ortho-phosphoric Acid 85% (Ph Eur, BP, NF)	Merck

Item	Manufacturer
1.5 mL glass cartridge	SFAM (siliconed at Aguettant)
Rubbers Type A	West Company
Crim caps	Aguettant
Millex-GV Syringe Driven Filter Unit – Durapore	Millipore
Durapore Membrane Filters 0.22 µm GV	Millipore
20 mL Plastic syringe Plastipak	Becton Dickinson
Steel Holder for filtration	Sartorius

Equipment

HPLC Systems	Detector mod. 486 or 490 Controller mod. 600S Pump mod. 626 Autosampler mod. 717	Waters	2
pH meter	Mod. 654	Metrohm	1
Osmometer	030-D	Osmomat	1

The following study evaluated the following parameters for a large number of formulations:

- Compatibility of surfactant and bacteriostatic
- Oxidation of alpha-subunit

The formulations were multi-dose formulations and contained either TWEEN 20 or Pluronic F68 as well as a bacteriostatic agent. The following three bacteriostatic agents were evaluated:

- Benzyl alcohol 0.9%
- *m*-Cresol 0.3%
- Phenol 0.5%

TWEEN 20 and Pluronic F68 were used at the following range of concentrations:

- TWEEN 20 : range from 10 to 100 µg/g
- Pluronic F68 : range from 10 to 100 µg/g

Solutions prepared are listed in Table 1.

Table 1: Comparative formulations							
ID #	Na_2HPO_4 $2H_2O$ (mg/g)	NaH_2PO_4 H_2O (mg/g)	r-hFSH*	Pluronic F68 (µg/g)	TWEEN 20 (µg/g)	Bacteriostat	Excipient (mg/g)
1P	1.11	0.45	600 IU/g	10	-	0.5% Phenol	Sucrose 70.6
2P	1.11	0.45	600 IU/g	10	-	0.5% Phenol	Mannitol 38.7
3P	1.11	0.45	600 IU/g	100	-	0.5% Phenol	Sucrose 70.6
4P	1.11	0.45	600 IU/g	100	-	0.5% Phenol	Mannitol 38.7
5P	1.11	0.45	600 IU/g	-	10	0.5% Phenol	Sucrose 70.6
6P	1.11	0.45	600 IU/g	-	10	0.5% Phenol	Mannitol 38.7
7	1.11	0.45	600 IU/g	-	100	0.9% benzyl alcohol	NaCl 6.0
8	1.11	0.45	600 IU/g	-	100	0.9% benzyl alcohol	Sucrose 62.3
9	1.11	0.45	600 IU/g	-	100	0.9% benzyl alcohol	Mannitol 34.1

Table 1: Comparative formulations							
ID #	Na_2HPO_4 $2H_2O$ (mg/g)	NaH_2PO_4 H_2O (mg/g)	r-hFSH*	Pluronic F68 (µg/g)	TWEEN 20 (µg/g)	Bacteriostat	Excipient (mg/g)
10	1.11	0.45	600 IU/g	-	100	0.3 % m-Cresol	NaCl 7.6
11	1.11	0.45	600 IU/g	-	100	0.3 % m-Cresol	Sucrose 78.0
12	1.11	0.45	600 IU/g	-	100	0.3 % m-Cresol	Mannitol 42.7
13	1.11	0.45	600 IU/g	-	10	0.9% benzyl alcohol	NaCl 6.0
14	1.11	0.45	600 IU/g	-	10	0.9% benzyl alcohol	Sucrose 62.3
15	1.11	0.45	600 IU/g	-	10	0.9% benzyl alcohol	Mannitol 34.1
16	1.11	0.45	600 IU/g	-	10	0.3 % m-Cresol	NaCl 7.6
17	1.11	0.45	600 IU/g	-	10	0.3 % m-Cresol	Sucrose 78.0
18	1.11	0.45	600 IU/g	-	10	0.3 % m-Cresol	Mannitol 42.7

Table 1: Comparative formulations							
ID #	Na_2HPO_4 $2H_2O$ (mg/g)	NaH_2PO_4 H_2O (mg/g)	r-hFSH*	Pluronic F68 (µg/g)	TWEEN 20 (µg/g)	Bacteriostat	Excipient (mg/g)
19	1.11	0.45	600 IU/g	100	-	0.9% benzyl alcohol	NaCl 6.0
20	1.11	0.45	600 IU/g	100	-	0.9% benzyl alcohol	Sucrose 62.3
21	1.11	0.45	600 IU/g	100	-	0.9% benzyl alcohol	Mannitol 34.1
22	1.11	0.45	600 IU/g	100	-	0.3% m-Cresol	NaCl 7.6
23	1.11	0.45	600 IU/g	100	-	0.3% m-Cresol	Sucrose 78.0
24	1.11	0.45	600 IU/g	100	-	0.3% m-Cresol	Mannitol 42.7
25	1.11	0.45	600 IU/g	10	-	0.9% benzyl alcohol	NaCl 6.0
26	1.11	0.45	600 IU/g	10	-	0.9% benzyl alcohol	Sucrose 62.3
27	1.11	0.45	600 IU/g	10	-	0.9% benzyl alcohol	Mannitol 34.1

Table 1: Comparative formulations							
ID #	Na_2HPO_4 $2H_2O$ (mg/g)	NaH_2PO_4 H_2O (mg/g)	r-hFSH*	Pluronic F68 (µg/g)	TWEEN 20 (µg/g)	Bacteriostat	Excipient (mg/g)
28	1.11	0.45	600 IU/g	10	-	0.3% m-Cresol	NaCl 7.6
29	1.11	0.45	600 IU/g	10	-	0.3% m-Cresol	Sucrose 78.0
30	1.11	0.45	600 IU/g	10	-	0.3% m-Cresol	Mannitol 42.7

*FSH was added to the formulations on the basis of its biopotency instead of protein content.

From visual examination of the formulations, it was determined that TWEEN 20 cannot be used with *m*-cresol and phenol because FSH formulations containing TWEEN 20 and *m*-cresol or TWEEN 20 and phenol presented a white opalescent suspension. In contrast, FSH formulations containing Pluronic F68 did not exhibit this problem with *m*-cresol and phenol. The use of Pluronic F68 permits the use of phenol and *m*-cresol.

Combination of FSH and Pluronic F68 with antioxidants

The following antioxidants were evaluated for their ability to inhibit oxidation of the α-subunit in the presence of Pluronic F68:

- Methionine : range from 10 to 100 µg/g
- Ascorbic Acid : range from 10 to 100 µg/g

Sucrose and Mannitol were used as tonicity agents and TWEEN 20 or Pluronic were added at the concentration of 100 µg/g.

The formulations prepared are listed in Table 2.

Table 2. Comparative formulations with and without methionine									
ID#	Na_2HPO_4 $2H_2O$ (mg/g)	NaH_2PO_4 H_2O (mg/g)	RhFSH	Pluronic F68 (µg/g)	TWEEN (µg/g)	Ascorbic Acid (µg/g)	Methionine (µg/g)	Bacteriostat	Excipient
31	1.11	0.45	600 IU/g	100	-	-	-	0.3% m-cresol	Sucrose
32	1.11	0.45	600 IU/g	100	-	-	-	0.3% m-cresol	Mannitol
33	1.11	0.45	600 IU/g	-	100	-	-	0.9% benzyl alcohol	Sucrose
34	1.11	0.45	600 IU/g	-	100	-	-	0.9% benzyl alcohol	Mannitol
35	1.11	0.45	600 IU/g	100	-	-	-	0.9% benzyl alcohol	Sucrose
36	1.11	0.45	600 IU/g	100	-	-	-	0.9% benzyl alcohol	Mannitol
37	1.11	0.45	600 IU/g	100	-	-	10	0.3% m-cresol	Sucrose
38	1.11	0.45	600 IU/g	100	-	-	10	0.3% m-cresol	Mannitol
39	1.11	0.45	600 IU/g	100	-	-	100	0.3% m-cresol	Sucrose

Table 2. Comparative formulations with and without methionine									
ID#	Na_2HPO_4 $2H_2O$ (mg/g)	NaH_2PO_4 H_2O (mg/g)	RhFSH	Pluronic F68 (µg/g)	TWEEN (µg/g)	Ascorbic Acid (µg/g)	Methionine (µg/g)	Bacteriostat	Excipient
40	1.11	0.45	600 IU/g	100	-	-	100	0.3% m-cresol	Mannitol
41	1.11	0.45	600 IU/g	100	-	10	-	0.3% m-cresol	Sucrose
42	1.11	0.45	600 IU/g	100	-	10	-	0.3% m-cresol	Mannitol
43	1.11	0.45	600 IU/g	100	-	100	-	0.3% m-cresol	Sucrose
44	1.11	0.45	600 IU/g	100	-	100	-	0.3% m-cresol	Mannitol
45	1.11	0.45	600 IU/g	-	100	-	10	0.9% benzyl alcohol	Sucrose
46	1.11	0.45	600 IU/g	-	100	-	10	0.9% benzyl alcohol	Mannitol
47	1.11	0.45	600 IU/g	-	100	-	100	0.9% benzyl alcohol	Sucrose
48	1.11	0.45	600 IU/g	-	100	-	100	0.9% benzyl alcohol	Mannitol

Table 2. Comparative formulations with and without methionine

ID#	Na_2HPO_4 $2H_2O$ (mg/g)	NaH_2PO_4 H_2O (mg/g)	RhFSH	Pluronic F68 (µg/g)	TWEEN (µg/g)	Ascorbic Acid (µg/g)	Methionine (µg/g)	Bacteriostat	Excipient
49	1.11	0.45	600 IU/g	-	100	10	-	0.9% benzyl alcohol	Sucrose
50	1.11	0.45	600 IU/g	-	100	10	-	0.9% benzyl alcohol	Mannitol
51	1.11	0.45	600 IU/g	-	100	100	-	0.9% benzyl alcohol	Sucrose
52	1.11	0.45	600 IU/g	-	100	100	-	0.9% benzyl alcohol	Mannitol
53	1.11	0.45	600 IU/g	100	-	-	10	0.9% benzyl alcohol	Sucrose
54	1.11	0.45	600 IU/g	100	-	-	10	0.9% benzyl alcohol	Mannitol
55	1.11	0.45	600 IU/g	100	-	-	100	0.9% benzyl alcohol	Sucrose
56	1.11	0.45	600 IU/g	100	-	-	100	0.9% benzyl alcohol	Mannitol
57	1.11	0.45	600 IU/g	100	-	10	-	0.9% benzyl alcohol	Sucrose

Table 2. Comparative formulations with and without methionine									
ID#	Na_2HPO_4 $2H_2O$ (mg/g)	NaH_2PO_4 H_2O (mg/g)	RhFSH	Pluronic F68 (µg/g)	TWEEN (µg/g)	Ascorbic Acid (µg/g)	Methionine (µg/g)	Bacteriostat	Excipient
58	1.11	0.45	600 IU/g	100	-	10	-	0.9% benzyl alcohol	Mannitol
59	1.11	0.45	600 IU/g	100	-	100	-	0.9% benzyl alcohol	Sucrose
60	1.11	0.45	600 IU/g	100	-	100	-	0.9% benzyl alcohol	Mannitol
61	1.11	0.45	600 IU/g	100	-	-	-	Phenol	Sucrose
62	1.11	0.45	600 IU/g	100	-	-	-	Phenol	Mannitol
63	1.11	0.45	600 IU/g					Phenol	Sucrose
64	1.11	0.45	600 IU/g	100	-	-	10	Phenol	Mannitol
65	1.11	0.45	600 IU/g	100	-	-	100	Phenol	Sucrose
66	1.11	0.45	600 IU/g	100	-	-	100	Phenol	Mannitol
67	1.11	0.45	600 IU/g	100	-	10	-	Phenol	Sucrose
68	1.11	0.45	600 IU/g	100	-	10	-	Phenol	Mannitol
69	1.11	0.45	600 IU/g	100	-	100	-	Phenol	Sucrose
70	1.11	0.45	600 IU/g	100	-	100	-	Phenol	Mannitol

FSH was added to the formulations on the basis of its biopotency instead of protein content.

20 g of each formulation was prepared into Falcon polypropylene tubes and filtered through a $3cm^2$ 0.22 µm Millex-GV Syringe Driven filter unit Durapore, then analysed for a value at t=0. The solutions were then stored at 40°C and tested according the following scheme:

Analytical test	T=0	1 week	2 weeks	3 weeks	4 weeks
Reverse Phase-HPLC for oxidised alpha subunit (%)	X	X	X	X	X
Size Exclusion-HPLC for protein quantitation (µg/g)	X	X	X	X	X
Size Exclusion-HPLC for qualitative free subunits	X	X	X	X	X

Reverse phase HPLC reveals that in formulations containing FSH, Pluronic F68, *m*-cresol and methionine (at 10 and 100 µg/ml), oxidation of the α-subunit of FSH when the formulation is stored at 40°C, is greatly reduced, versus a formulation containing no methionine, as can be seen in Figure 1. Based on the average of two experiments, in the Formulation containing no methionine, the percent of oxidised α-subunit is 2.3 at T=0; 4.0 at T= 1 week, and 7.1 at T= 2 weeks. In the formulation containing 10 µg/ml methionine, the percent of oxidised α-subunit is 2.0 at T=0, 3.2 at T= 1 week, and 3.8 at T= 2 weeks. In the formulation containing 100 µg/ml methionine, the percent of oxidised α-subunit is 1.8 at T=0, 1.7 at T= 1 week, and 1.3 at T= 2 weeks.

Formulations containing Ascorbic acid became yellow when stored at 40 °C, and were judged to be unacceptable.

Example 2

Liquid single-dose formulation of recombinant FSH for subcutaneous or intramuscular injection

Based on the results of Example 1, the following formulation was prepared.

Components 1 to 7 listed in Table 3 were mixed as solids in a vial and dissolved in WFI to the given volume. The resulting formulations deliver a single dose of 150 or 75 IU FSH.

With recombinant FSH, the bioactivity and specific activity are consistent, allowing the FSH to be filled by mass, rather than by bioassay.

Table 3. Components of FSH single dose liquid formulations

Component #	Description	150 IU FSH	75 IU FSH
1	rhFSH (µg/vial)	10.9 (150 IU)	5.45 (75 IU)
2	Sucrose (mg/vial)	15.00	7.50
3	$NaH_2PO_4 \cdot H_2O$ (mg/vial)	0.111	0.0555
4	$Na_2HPO_4 \cdot 2H_2O$ (mg/vial)	0.273	0.1365
5	Pluronic F68 (mg/vial)	0.025	0.0125
6	Methionine (mg/vial)	0.025	0.0125
7	*m*-cresol (mg/vial)	0.75	0.375
8	PH	7.0	7.0
9	WFI	q.s. to 1 ml	q.s. to 0.5 ml

The vials were filled and sealed under sterile conditions. The formulation has a shelf life of up to two years.

Example 3

Liquid multi-dose formulation of recombinant FSH for subcutaneous or intramuscular injection

Based on the results of Example 1, the following multi-dose formulation was prepared.

Components 1 to 7 listed in Table 4 were mixed as solids in a cartridge and dissolved in WFI to the given volume. The resulting formulations deliver a total of 300, 450 and 900 IU of FSH.

The cartridges were filled and sealed under sterile conditions. The multi-dose formulation can be stored at room temperature (25 °C) until the first use for up to two years. After the first use, the cartridge should be stored at at or about 5 °C, over the multi-dose period, which may be 24 hours, 2 days, or up to 12 days.

Table 4. Components of FSH multi-dose liquid formulations				
Component #	Description	300 IU FSH	450 IU FSH	900 IU FSH
1	rhFSH (µg/cartridge)	22.2 (305 IU)	33.3 (458 IU)	66.7 (916 IU)
2	Sucrose (mg/cartridge)	30.0	45.0	90.0
3	$NaH_2PO_4 \cdot H_2O$ (mg/cartridge)	0.225	0.337	0.675
4	$Na_2HPO_4 \cdot 2H_2O$ (mg/cartridge)	0.555	0.832	1.665
5	Pluronic F68 (mg/vial)	0.050	0.075	0.150
6	Methionine (mg/vial)	0.050	0.075	0.150
7	*m*-cresol (mg/vial)	1.50	2.25	4.50
8	pH	7.0	7.0	7.0
9	WFI	q.s. to 0.5 ml	q.s. to 0.75 ml	q.s. to 1.5 ml

Sequences:

SEQ ID NO. 1: hFSH α-subunit;

SEQ ID NO. 2: hFSH β-subunit

SEQ ID NO. 3: hFSH β-subunit variant 1

SEQ ID NO. 4: hFSH β-subunit variant 2

SEQ ID NO. 5: hFSH β-subunit variant 3

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Claims

1. A liquid pharmaceutical composition comprising follicle-stimulating hormone or a variant thereof, and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

2. A pharmaceutical composition according to claim 1, wherein the surfactant is Pluronic F68.

3. A pharmaceutical composition according to claim 1, wherein the follicle-stimulating hormone is human follicle-stimulating hormone.

4. A pharmaceutical composition according to claim 1, wherein the follicle-stimulating hormone is urinary human follicle-stimulating hormone.

5. A pharmaceutical composition according to claim 1, wherein the follicle-stimulating hormone is recombinant human follicle-stimulating hormone.

6. A pharmaceutical composition according to any one preceding claim, wherein the follicle-stimulating hormone is present at a concentration of at or about 150 IU/ml to at or about 1'200 IU/ml.

7. A pharmaceutical composition according to any one preceding claim, wherein the follicle-stimulating hormone is present at a concentration of at or about 300 IU/ml to at or about 900 IU/ml.

8. A pharmaceutical composition according to any one preceding claim, wherein the follicle-stimulating hormone is present at a concentration of at or about 600 IU/ml.

9. A pharmaceutical composition according to any one preceding claim, further comprising a bacteriostatic selected from phenol and *m*-cresol.

10. A pharmaceutical composition according to claim 9, wherein the bacteriostatic is *m*-cresol.

11. A pharmaceutical composition according to any one preceding claim, comprising *m*-cresol at a concentration of at or about 0.3% (mass/mass solvent).

12. A pharmaceutical composition according to any one preceding claim, further comprising sucrose.

13. A pharmaceutical composition according to any one preceding claim, further comprising sucrose at a concentration that is calculated according to equation (3):

$$\text{Amount of sucrose} = \frac{\text{Total FSH in IU}}{\text{10 IU/mg}} \qquad (3)$$

14. A pharmaceutical composition according to any one preceding claim, further comprising methionine.

15. A pharmaceutical composition according to any one preceding claim, further comprising methionine in an amount that is calculated according to equation (2):

$$\text{Amount of methionine} = \frac{\text{Total FSH in IU}}{\text{6'000 IU/mg}} \qquad (2)$$

16. A pharmaceutical composition according to any one preceding claim, further comprising phosphate buffer at a pH of at or about 6.0 to at or about 8.0.

17. A pharmaceutical composition according to any one preceding claim, further comprising phosphate buffer at a pH of at or about 7.0.

18. A pharmaceutical composition according to any one preceding claim, for multi-dose use.

19. A pharmaceutical composition according to claim 1, in liquid form for multi-dose use, comprising the following ingredients: rFSH, Pluronic F68, sucrose,

methionine, *m*-cresol, and phosphate buffer at a pH of at or about 7.0, dissolved in water for injection.

20. A pharmaceutical composition according to claim 19, wherein the rFSH is present at a concentration of at or about 600 IU/ml, the Pluronic F68 is present at a concentration of at or about 0.1 mg/ml, the sucrose is present at a concentration of at or about 60 mg/ml, the methionine is present at a concentration of at or about 0.1 mg/ml, the *m*-cresol is present at a concentration of at or about 3 mg/ml, and the phosphate buffer is at or about 10 mM in phosphate.

21. A pharmaceutical composition according to any one preceding claim, for subcutaneous or intramuscular injection.

22. A pharmaceutical composition according to any one preceding claim, for subcutaneous injection.

23. A method for manufacturing a liquid pharmaceutical composition comprising forming a solution of FSH, and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and a liquid diluent.

24. A method according to claim 23, wherein the surfactant is Pluronic F68.

25. A method according to claim 23 or 24, comprising the further step of adding a bacteriostatic selected from phenol and *m*-cresol.

26. A method for manufacturing a packaged pharmaceutical composition comprising placing a solution comprising FSH, and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, in a vial, ampoule or cartridge.

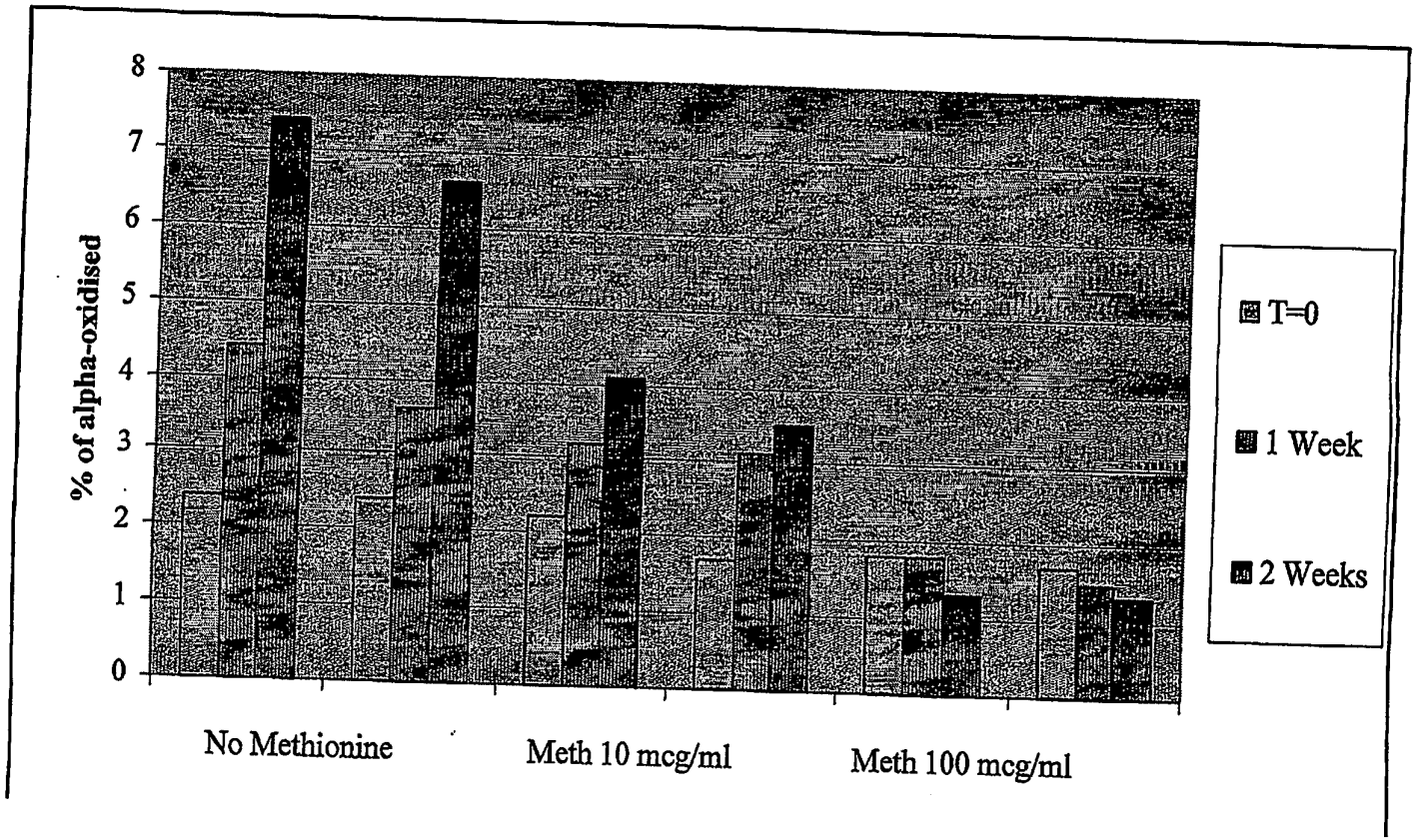
27. A method according to claim 26, wherein the surfactant is Pluronic F68.

28. A method according to claim 26 or 27, wherein the solution additionally comprises a bacteriostatic selected from phenol and *m*-cresol.

Abstract

Provided are stable liquid formulations of FSH with a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

Figure 1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6
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